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The influence of microbial synergistic and antagonistic effects on the performance of refinery wastewater microbial fuel cells

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HIGHLIGHTS

- Microbial synergistic and antagonistic effect does exist among diverse microbial strains in MFC.
- Microbial synergistic and antagonistic effect can directly influence key performances of MFC.
- Microbial synergistic effect improves performance of MFC but antagonistic effect degrades it.
- Petroleum hydrocarbons degradation by different microbial strains is different.

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ABSTRACT

This study provides a preliminary investigation of the synergistic and antagonistic effects of different microbial strains and their influence on electricity generation and wastewater treatment performances in microbial fuel cells (MFCs). Microbial metabolic characteristics of petroleum hydrocarbon pollutants are studied simultaneously to provide further insight into how microbial synergistic and antagonistic effects influence MFCs. We observed a synergistic effect between *Paenibacillus sp.* and *Deinococcus sp.* and an antagonistic effect between *Microbacterium sp.* and *Paenibacillus sp.* and *Deinococcus sp.* The microbial synergistic and antagonistic effects significantly influenced MFC performance directly. The best MFC performance was observed with *Paenibacillus sp.* + *Deinococcus sp.* due to their synergistic effect, where the power density output reached 102.93 mW m^{-3}, and the oil removal rate was 85.56 ± 1.10%. However, the performances of MFCs inoculated with *Microbacterium sp.* were considerably poorer because of its antagonistic effect on the other microbial strains, where the lowest power density output was 24.93 mW m^{-3}, and the oil removal rate was 65.88 ± 1.10%. The degradation characteristics of petroleum hydrocarbons differ between microbial strains; thus, the relative results can provide further insight into how microbial synergistic and antagonistic effects influence MFCs.

1. Introduction

A large number of chemical bond energy is stored in wastewater. Researchers have demonstrated that at least 2.2×10^{18} J of chemical bond energy is stored in municipal wastewater generated in the world every year, which is equivalent to approximately 70 GW of electrical energy and 52 million tons of oil [1]. This estimate is based on chemical bond energy stored in municipal wastewater alone and does not include agricultural wastewater and industrial wastewater. Therefore, chemical bond energy recovery together with wastewater treatment can be significant and could be applied to solve global issues, such as energy shortage and environmental pollution [2,20].

A microbial fuel cell (MFC) is a special fuel cell device that uses electricigens as a cheap anode catalyst. MFCs can directly convert chemical bond energy to electrical energy together with the purification of wastewater [3]. One of the key factors limiting the practical industrial application of MFC technology is the low power output and energy recovery efficiency [4–7]. How to improve the electricity generation and energy recovery performance is an important subject among MFC research fields. Various factors can influence the MFC performance, such as reactor configuration [8], electrode material [9], separating medium [10], anolyte pH [11], external resistance [12], and temperature [13]. Among them, electricigens are one of the most important factors that influence MFCs. To date, electricigen studies have focused on testing and improving the electricity generation performance of electricigen strains [14],

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screening new electricigen strains [15], microbial population and diversity evolution [16] and exploring the electricity generation mechanism of microbial strains [17]. However, detailed studies on the microbial synergistic and antagonistic effects and their influence on MFC performance remain lacking. Hassan et al. [18] attempted to use mixed and pure cultures of cellulose-degrading electricity generating bacteria from insoluble cellulose and demonstrated that in pure culture, the synergistic effect between cellobiose enzymes and pure bacteria was essential to generate electricity, but the synergistic effect between the different bacterial strains was not studied.

Refinery wastewater generated from petroleum processing contains plenty of organic pollutants, such as petroleum hydrocarbons, which are toxic to microbes and are poorly biodegraded [19]. The total emissions from refinery wastewater in China reach 500 million ta^{-1}. To date, traditional physicochemical and biological methods have been used most widely to treat refinery wastewater, but a large number of energy is consumed during the treatment process. When MFC technology is used to treat refinery wastewater, not only is the energy consumption of the traditional treatment process reduced but also the chemical bond energy stored in wastewater can also be recovered [2,20]. Therefore, it represents a viable pathway for wastewater resource utilization.

In this study, microbial strains were separated from a double-chambered refinery wastewater microbial fuel cell, which was stably operated for one year, and were used to inoculate MFCs to investigate the synergistic and antagonistic effects of different microbial strains and their influence of the electricity generation and wastewater treatment performances of MFCs. We investigated the microbial metabolic characteristics of petroleum hydrocarbon pollutants simultaneously to provide further insight into how the microbial synergistic and antagonistic effects influence MFCs.

2. Materials and methods

2.1. MFC construction

The configuration of MFC used in this study is presented in Fig. 1. It was made from glasses, and the volume of the two chambers was 400 mL. The anode chamber was sealed to maintain an anaerobic environment, and 100 mL of graphite granule or activated carbon granule was used as the packing materials (average grain diameter of 0.5–2.0 mm and porosity of 0.44). A graphite rod was used for the anode (0.6 cm × 18 cm). The cathode chamber was aerated continuously to maintain a constant dissolved oxygen concentration, a graphite plate was used as a cathode, and 20 mM Fe(III)-EDTA, which was a regenerable cathodic electron acceptor, was used as a catholyte [21]. The two chambers were separated by a proton exchange membrane (Nafion 117) and connected with a flange plate. The external resistance was 1000 Ω, and the operation temperature was 30 °C when unspecified.

2.2. Anolyte

The MFC anolyte was a mixture of refinery wastewater and phosphate buffer (1:1). The phosphate buffer was prepared refer following a previously published protocol [22], whereas the refinery wastewater was collected from the effluent of the flotation process in the Beijing Yanshan Refinery. The chemical oxygen demand of anolyte was 250 ± 40 mg L^{-1}, the oil concentration was 17 ± 1 mg L^{-1} and the pH was 7.1 ± 0.1. The anolyte was aerated with N_2 for 30 min to maintain an anaerobic environment.

2.3. Calculations

The MFC voltage output was recorded automatically with a data logger (e-corder, ED401, eDAQ Pty. Ltd, Australia) at 1 min intervals. The current density and power density was calculated with the following formulas:

$$I = UR^{-1}V^{-1} \quad (1)$$

$$P = UI \quad (2)$$

where I is the current density (mW m^{-3}), U is the voltage (mV), R is the external resistance (Ω), V is the working volume of the anode chamber (m^3) and P is the power density (mW m^{-3}).

The apparent internal resistance was determined according to the steady state discharge method [23].

2.4. Analytics

The chemical oxygen demand (COD) was measured by a 5B-6 COD speed meter (LianHua Tech, Lanzhou, China), the oil concentration was determined using an infrared oil analyzer (MC-OIL420, HuaxiaKechuang, Inc., China) after carbon tetrachloride extraction and the pH was determined with a pH monitor (PHSJ-4, Leici Instrument, Inc., Shanghai, China).

The organic composition of the refinery wastewater was analyzed by GC–MS. First, samples extracted from the wastewater by guarantee reagent methylene chloride were injected into a commercial quadrupole mass spectrometer (SSQ-710C, Thermo-Finnigan, San

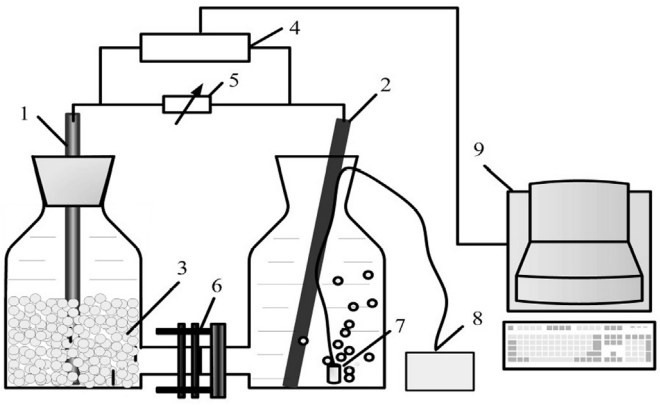

Fig. 1. Schematic diagram of the MFC double chambers. 1 anode; 2 cathode; 3 activated carbon; 4 data recorder; 5 resistance box; 6 PEM; 7 aerator; 8 air pump; 9 computer.

Jose, California) connected to a Varian 3400 GC (Varian, Middleburg, the Netherlands). A septum equipped temperature programmable injector (SPI) was used together with a DB-5 HT capillary column (15 m × 0.25 mm × 0.1 μm, J&W Scientific, Folsom, USA). The temperature program for the GC capillary column was 60 °C, which was maintained for 10 min and then, raised to 300 °C at a rate of 8 °C min^{-1} and maintained for 20 min. Electron capture negative ionization (ECNI) with methane (scientific 5.5, AGA Stockholm, Sweden) as a reagent gas and an electron energy of 70 eV was used. Helium was used as the carrier gas, the ion source temperature was 200 °C and the transfer line temperature was set to 290 °C. The selected ion monitoring (SIM) mode (isotopes mz^{-1} 79 and 81) was used.

2.5. Microbial strain isolation and 16S rDNA amplification

Microbial strains were isolated and purified using the streak plate method. The packing materials in each MFC were transferred to sterile liquid culture medium, which contained 0.5 g L^{-1}yeast extract, 0.5 g L^{-1}proteose peptone, 0.5 g L^{-1}casamino acids, 0.5 g L^{-1} glucose, 0.5 g L^{-1} solution starch, 0.3 g L^{-1} K_2HPO_4, 0.05 g L^{-1} $MgSO_4 \cdot 7H_2O$ and 0.3 g L^{-1} sodium pyruvate, using a sterile lab spoon and incubated in a bed temperature incubator (BHWY-200, Saifu, China) for 48 h to achieve enlarge cultivation. Then the bacterial culture medium and its dilutions were streaked onto agar plates containing the culture media that was used in the enlarge cultivation and incubated at 30 °C. The bacterial colonies that formed on the plates were picked and further purified by re-streaking on new agar plates. A small amount of the purified bacterial cells was then picked using a needle and suspended in PBS solution for 16S rDNA extraction using the fast DNA SPIN kit for soil (MP BIO, IIIKirch, France). The 16S rDNA of the bacterial strains was amplified using broad-range bacterial primers Bact-8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and Bact-1492R (5′GGTTACCTTGTTACGA CTT 3′). The following reagents were used: 5.0 μL 10× PCR buffer II (Applied Biosystems, Foster City, CA), 3.0 μL 25 mM $MgCl_2$ (Applied Biosystems), 2.5 μL 1%Triton X-100, 4.0 μL 250 mM tetramethylammonium chloride, 2.0 μL 10 mM deoxyribonucleoside triphosphates, 1.0 μL forward primer and 1.0 μL reverse primer (20 pmol μL^{-1} each), 0.5 μL AmpliTaq DNA polymerase (5 U μL^{-1}; Applied Biosystems, Foster City, CA) and 5.0 μL of 1:10 diluted template DNA in a total volume of 50 μL. PCR was performed using a GeneAmp PCR System 9700 cycler (Applied Biosystems, Foster City, CA). The following cycling parameters were used: 5 min for an initial denaturation at 95 °C followed by 20 cycles for denaturation (30 s at 95 °C), annealing (30 s at 56 °C), and elongation (90 s at 72 °C), with a final extension at 72 °C for 8 min. The PCR products were sequenced, and then the sequences were compared directly to all known sequences deposited in the GenBank NCBI database (http://www.ncbi.nlm.nih.gov) using the basic local alignment search tool (BLAST).

3. Results and discussion

3.1. Object microbial strain screening of preliminary experiments

Dominant microbial strains enriched on the activated carbon and graphite granules in the anode chamber of double-chambered refinery wastewater MFCs were separated and purified using the streak plate method (described in Section 2.5), and the single strains were analyzed using the 16S rDNA method. The results revealed that the MFCs from activated carbon granules harbored three dominant microbial strains: *Microbacterium sp.*, *Bacillus sp.* and *Acinetobacter sp.* The MFC graphite granule harbored six dominant microbial strains: *Microbacterium sp.*, *Bacillus sp.*, *Nicotinic acid bacillus sp.*, *Paenibacillus sp.*, *Deinococcus sp.* and *Arthrobacter sp.* Among them, *Microbacterium sp.*, *Nicotinic acid bacillus sp.* and *Paenibacillus sp.* are facultative anaerobes, *Bacillus sp.* is an amphimicrobe and *Acinetobacter sp.*, *Deinococcus sp.* and *Arthrobacter sp.* are facultative aerobes.

To screen microbial strains that exhibit good electricity generation performances for a microbial co-metabolic study, these eight single microbial strains were separated from double-chambered refinery MFCs and were used to inoculate new refinery MFCs. These MFCs inoculated with single microbial strains were continuously cultivated to maintain a stable operation period. The results demonstrated that the MFCs inoculated with eight single microbial strains yielded the highest voltage output, where 42 days after the MFC was turned on, the highest voltage output was higher than 200.00 mV, and after 4 months of continuous cultivation, the highest voltage output reached 315.40 mV. Compared to this MFC, the voltage outputs of MFCs inoculated with a single microbial strain were lower. MFCs inoculated with *Paenibacillus sp.* performed the best, where the highest voltage output was 270.16 mV, followed by *Microbacterium sp.* 2 (which was separated from the MFC graphite granule), *Deinococcus sp.*, *Nicotinic acid bacillus sp.*, *Microbacterium sp.* 1 (which was separated from the MFC activated carbon granule), *Bacillus sp.*, *Arthrobacter sp.* The voltage output of MFCs inoculated with *Acinetobacter sp.* was the lowest, where the highest voltage output was 162.96 mV. According to the electricity generation performances of each MFC, the three microbial strains, *Microbacterium sp.*, *Paenibacillus sp.* and *Deinococcus sp.*, that yielded better electricity generation performances were chosen for further study using orthogonal experiments to explore the synergistic and antagonistic effects and metabolic characteristics of different microbial strains in refinery wastewater MFCs.

According to the preliminary experiments, the electricity generation performance of MFCs inoculated with eight microbial strains was better than MFCs inoculated with a single microbial strain, but its voltage output was still lower than MFCs inoculated with activated sludge from refinery wastewater treatment systems. This result indicated that a microbial synergistic effect existed between different dominant microbial strains in MFCs, and a synergistic effect also existed between dominant microbial strains and non-dominant microbial strains that could not be separated from protozoans that are present in activated sludge. To study the microbial synergistic and antagonistic effects, *Microbacterium sp.*, *Paenibacillus sp.* and *Deinococcus sp.*, which displayed better electricity generation performances, were selected as object microbial strains based on the preliminary electricity generation experiments using a single microbial strain and microbial strains characteristics for the following orthogonal experiments.

3.2. Orthogonal analysis of object microbial strains

To study the microbial synergistic and antagonistic effects and metabolic characteristics further, *Microbacterium sp.*, *Paenibacillus sp.* and *Deinococcus sp.* were selected as object microbial strains (described in Section 3.1), and the orthogonal experiments were performed as illustrated in Table S1. Single microbial strains inoculated in the MFCs were pure cultured and detected at an OD_{600} to determine their growth conditions. According to the OD_{600} data and the inoculum volume, when the MFC was inoculated with more than one microbial strain, the quantity of each single microbial strain was equal to the inoculum. Seven MFC groups were constructed and inoculated with three single microbial strains, and their mixtures are presented in Table S1. These MFCs were cultivated continuously at the same conditions, and the anolyte was replaced every 5 days. When the MFCs were operated stably, the microbial synergistic effects and metabolic characteristics were analyzed.

3.3. Initiation and stable operation of orthogonal experiments

The voltage generation performance of each MFC orthogonal experiment is presented in Figs. Sa–Sg. The best voltage output performance was obtained in MFC-P + D (Fig. Sf), the start-up voltage reached 236.31 mV and then decreased to 150.00 mV. After 20 days of continuous cultivation, the highest voltage output reached 250.00 ± 10.00 mV and was maintained stably. The sub-optimum voltage output performance was achieved in MFC-P (Fig. Sb), where the star-up voltage was 187.94 mV, which decreased slowly. Under continuous cultivation with MFC-P + D for 21 days, MFC-Preached a stable operation period with a maximum voltage output of 234.00 ± 10.00 mV. The voltage output performances of other MFCs are listed in the following descending order: S:MFC-M (Fig. Sa), MFC-D (Fig. Sc), MFC-M + D (Fig. Se), MFC-M + P + D (Fig. Sg) and MFC-M + P (Fig. Sd). The performance of MFC-M + P (Fig. Sd) was the worst, where the start-up voltage was 133.87 mV, and after 21 days of continuous cultivation, the highest voltage output was still lower than 140.00 mV. In Figs. Sa–Sg, after the MFCs were operated for 500 h, electricity was produced without a lag phase when the anolyte was replaced. The initial lag phase appeared at the initial incubation and then disappeared when the MFCs were started up, indicating that the electricity generated from refinery wastewater was primarily due to direct electron transfer by each bacteria attached to the anode and did not require an accumulation of mediators in the fresh solution [18].

According to voltage output of each MFC, the performance of MFC-P + D inoculated with *Paenibacillus sp.* + *Deinococcus sp.* was observably better than other MFCs, indicating that the microbial synergistic effect indeed existed between *Paenibacillus sp.* and *Deinococcus sp.*, whereas the performance of MFC-M + P inoculated with *Microbacterium sp.* + *Paenibacillus sp.* was much worse than the other MFCs. The performance of MFC-M + D inoculated with *Microbacterium sp.* + *Deinococcus sp.* and the performance of MFC-M + P + D inoculated with each of the three microbial strains was also worse than MFCs inoculated with *Paenibacillus sp.* (MFC-P), *Microbacterium sp.* (MFC-M) or *Deinococcus sp.* (MFC-D). This demonstrated that *Microbacterium sp.* inhibited the microbial activity of *Paenibacillus sp.* and *Deinococcus sp.*, and thus, a microbial antagonistic effect was apparent between them. Further, the depressant effect of *Microbacterium sp.* on *Paenibacillus sp.* was observably stronger than that of *Deinococcus sp.*, so the performance of MFCs inoculated with *Microbacterium sp.* + *Paenibacillus sp.* was the worst. A microbial synergistic effect between *Paenibacillus sp.* and *Deinococcus sp.* was weaker than the antagonistic effect generated from *Microbacterium sp.*, so the performance of MFCs inoculated with all three strains was slightly better than MFCs inoculated with *Microbacterium sp.* and *Paenibacillus sp.*, but observably worse than the other MFCs.

3.4. The influence of microbial synergistic and antagonistic effects on MFC electrochemical performance

To verify the synergistic and antagonistic effects observed in Section 3.3, the electrochemical performance of each MFC was investigated. When MFCs were operated in stable periods, their external resistances were changed continuously, and the voltage outputs together with the anode potentials were detected to draw the MFC polarization curves (Fig. 2a and b). The results demonstrated that the maximum power density output of MFC-M, MFC-P, MFC-D, MFC-M + P, MFC-M + D, MFC-P + D and MFC-M + P + D was 73.86 mW m^{-3}, 85.41 mW m^{-3}, 67.78 mW m^{-3}, 24.93 mW m^{-3}, 53.34 mW m^{-3}, 102.93 mW m^{-3} and 44.42 mW m^{-3},respectively, whereas the lowest anode potentials were −20.90 mV, −31.35 mV, −18.53 mV, 71.47 mV, 7.06 mV, −50.81 mV and 23.17 mV, respectively. According to Fig. 2c, the anode potential gradients that increased with the current density were 0.0117 mV mA^{-1} m^{-3}, 0.0102 mV mA^{-1} m^{-3}, 0.0152 mV mA^{-1} m^{-3}, 0.1542 mV mA^{-1} m^{-3}, 0.0525 mV mA^{-1} m^{-3}, 0.0038 mV mA^{-1} m^{-3} and 0.066 mV mA^{-1} m^{-3}.

The maximum power density output of MFC-P + D inoculated with *Paenibacillus sp.* + *Deinococcus sp.* was the highest (102.93 mW m^{-3}), followed by MFC-P inoculated with *Paenibacillus sp.* (85.41 mW m^{-3}) and the other MFCs in decreasing order: MFC-M inoculated with *Microbacterium sp.*, MFC-D inoculated with *Deinococcus sp.*, MFC-M + D inoculated with *Microbacterium sp.* + *Deinococcus sp.*, MFC-M + P + D inoculated with all three microbial strains and MFC-M + P inoculated with *Microbacterium sp.* + *Paenibacillus sp.* (73.86 mW m^{-3} > 67.78 mW m^{-3} > 53.34 mW m^{-3} > 44.42 mW m^{-3} > 24.93 mW m^{-3}). This was consistent with the voltage output performance of each MFC, further demonstrating that the presence of a microbial synergistic effect between *Paenibacillus sp.* and *Deinococcus sp.* would improve their electricity generation performance, whereas the microbial antagonistic effect generated by *Microbacterium sp.* to *Deinococcus sp.* and *Deinococcus sp.* would inhibit the electricity generation performance of these microbial strains.

When MFCs were operated in stable periods, the apparent internal resistance of each MFC was determined with the steady state discharge method. The results revealed that this resistance correlated with the voltage and power density output performance, and the internal resistance of MFCs inoculated with *Microbacterium sp.* + *Paenibacillus sp.* was the highest (2149.0 Ω), followed by MFCs inoculated with all three microbial strains (1423.0 Ω) and the other MFCs in descending order: MFCs inoculated with *Microbacterium sp.* + *Deinococcus sp.*, *Deinococcus sp.*, *Microbacterium sp.*, *Paenibacillus sp.* and *Paenibacillus sp.* + *Deinococcus sp.* (1386.0 Ω > 1365.0 Ω > 1355.7 Ω > 1321.3 Ω > 859.0 Ω). The apparent internal resistance of MFC is constituted by ohmic resistance and non-ohmic resistance and non-ohmic resistance, which can further be divided into activated internal resistance and mass transfer internal resistance. Ohmic resistance is caused by the inhibition from cell components and electrolytes from the transfer of electrons and protons. It is an important and inherent section of MFC internal resistance, where the activated internal resistance is caused by an activation energy barrier that should be surmounted during the electrochemical reaction. The mass transfer internal resistance is caused by inhibition effect caused by the diffusion of the reactant and metabolite to the electrode and solution-surfaces [3]. When the configuration of the MFC, electrolyte, operating conditions and substrates were the same, the apparent internal resistance difference was caused only by the transmission resistance of the electrons generated by the microbial metabolic reaction to the anode. Therefore, the synergistic and antagonistic effects of microbes would directly influence the electron transfer rate and the apparent internal resistance of MFCs. When MFCs were inoculated with *Paenibacillus sp.* + *Deinococcus sp.*, the microbial synergistic effect promoted their electron transfer performance and reduced the effective transmission resistance of electrons to the anode, and thus yielded the lowest apparent internal resistance. However, *Microbacterium sp.* exerted an antagonistic effect against *Paenibacillus sp.* and *Deinococcus sp.*, which inhibited the electron transfer activity of these microbial strains, so the relative internal resistance was high.

In addition to the power density output and apparent internal resistance, the microbial synergistic and antagonistic effects could also influence the polarization level that changes along with the current density. When the MFC-M + P was inoculated with *Microbacterium sp.* + *Paenibacillus sp.*, along with an increase in the current density, the polarization phenomenon of the anode was the most striking, followed by that of the MFC-M + P + D inoculated with all three microbial strains, and the polarization phenomenon

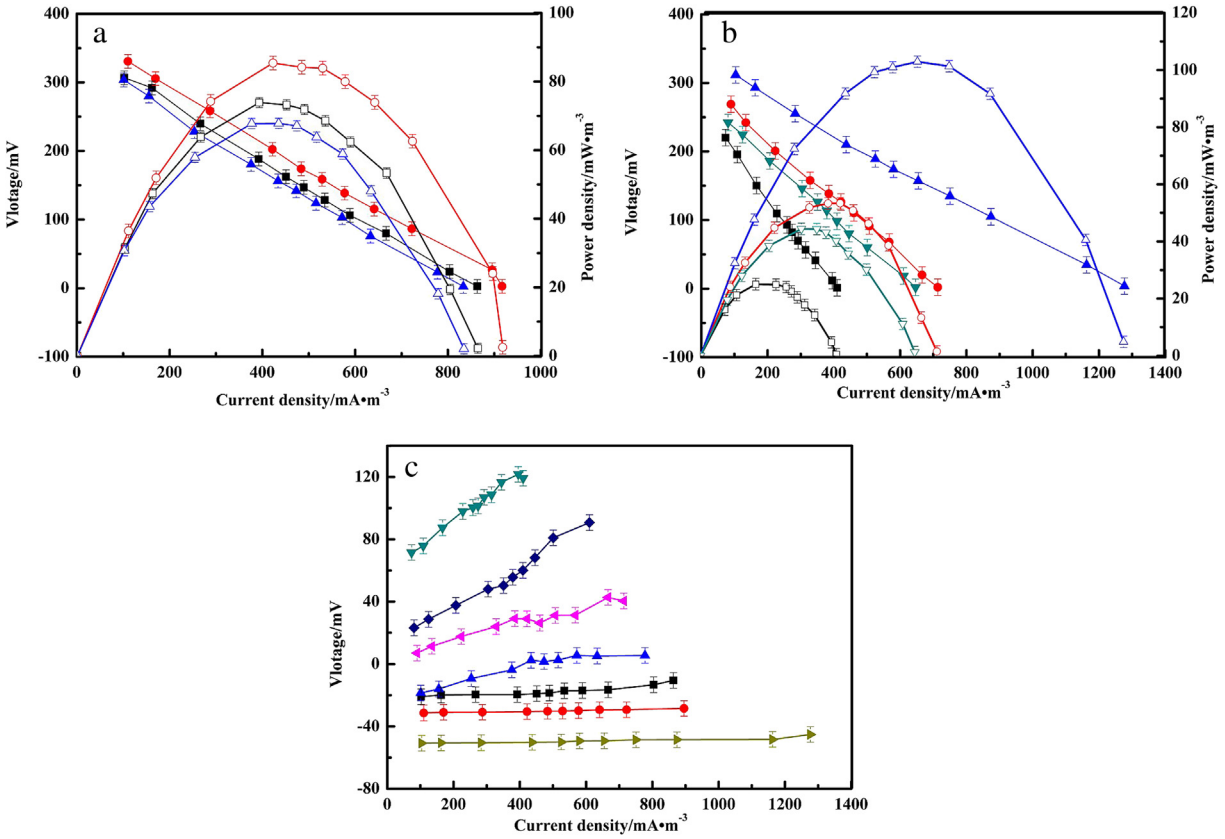

Fig. 2. a. The polarization curve of an MFC inoculated with *Microbacterium sp.* (MFC-M), *Paenibacillus sp.* (MFC-P) and *Deinococcus sp.* (MFC-D). (● Voltage of MFC-P; ■ Voltage of MFC-M; ▲ Voltage of MFC-D; ○ Power density of MFC-P; □ Power density of MFC-M; △ Power density of MFC-D). b. The polarization curve of an MFC inoculated with *Microbacterium sp.* + *Paenibacillus sp.* (MFC-M + P), *Microbacterium sp.* + *Deinococcus sp.* (MFC-M + D), *Paenibacillus sp.* + *Deinococcus sp.*(MFC-P + D) and *Microbacterium sp.* + *Paenibacillus sp.* + *Deinococcus sp.*(MFC-M + P + D). (▲ Voltage of MFC-P + D; ● Voltage of MFC-M + D; ▼ Voltage of MFC-M + P + D; ■ Voltage of MFC-M + P; △ Power density of MFC-P + D; ○ Power density of MFC-M + D; ▽ Voltage of MFC-M + P + D; □ Power density of MFC-M + P). c. The anode potential curves of each MFC (■ Anode potential of MFC-M; ● Anode potential of MFC-P; ▲ Anode potential of MFC-D; ▼ Anode potential of MFC-M + P; ◄ Anode potential of MFC-M + D; ► Anode potential of MFC-P + D; ◆ Anode potential of MFC-M + P + D).

of the anode in MFC-P + D inoculated with *Paenibacillus sp.* + *Deinococcus sp.* was the smallest. The result is due to the antagonistic effect that was generated between the *Microbacterium sp.* in the MFC and the other two strains, which inhibited their electron transfer activity and increased the electron transmission resistance. Therefore, when the external resistance was reduced, electrons could not be transferred in a timely manner and instead accumulated near the anode, generating an overpotential and a notable polarization phenomenon. Similarly, the lowest polarization phenomenon in the MFC-P + D inoculated with *Paenibacillus sp.* + *Deinococcus sp.* was attributed to the microbial synergistic effect that promoted the electron transfer between them.

3.5. The influence of microbial synergistic and antagonistic effects on MFC wastewater treatment performance

To explore the influence of microbial synergistic and antagonistic effects on the wastewater treatment efficiencies of MFCs, 7 days after MFCs had reached a stable operation period, the COD and oil concentrations of the MFC wastewater influent and effluent were tested 6 times to ensure the accuracy of the results, which are displayed in Table 1. The wastewater treatment efficiency of MFC-P + D inoculated with *Paenibacillus sp.* + *Deinococcus sp.* was the best, the removal rate of the COD reached 87.00 ± 1.10%, and the relative oil removal rate was 85.56 ± 1.10%, also the highest. The wastewater treatment efficiency of the MFC-M + P inoculated with *Microbacterium sp.* + *Paenibacillus sp.* was the worst, the removal rate of the COD was 76.13 ± 1.10% and the oil relative removal rate was 65.88 ± 1.10%, also the worst.

According to the results, the wastewater treatment efficiency could be influenced by the microbial synergistic and antagonistic effects directly. Because the microbial synergistic effect that existed between *Paenibacillus sp.* and *Deinococcus sp.* could promote the electron transfer rate (the apparent internal resistance of MFC-P + D was the lowest; 859.0 Ω), the electrons generated from the microbial metabolic reaction could be transferred to the anode surface in a timely manner and the degradation reaction rate of petroleum hydrocarbons would be facilitated. Therefore, the removal rate of pollutants was improved. However, in MFCs inoculated with *Microbacterium sp.* together with other strains, the microbial electron transfer performance was inhibited by the microbial antagonistic effects generated from *Microbacterium sp.* (the apparent internal resistance of these MFCs was all higher than 859.0 Ω). Because the electron transfer process was blocked, the electrons accumulated around the anode, restraining the degradation reaction rate of petroleum hydrocarbons, so the removal rate of pollutants was lowered.

Table 1 illustrates that there are few differences among the COD removal rates of the MFCs, whereas the oil concentration difference is much bigger. This may be because in MFCs with better electron transfer performance, the rate of the microbial metabolic reaction was faster, and a great quantity of metabolites, such as acids and esters, were generated. This inference was evidenced by the pH change of the anolyte in different MFCs. In MFCs with better

Table 1
The wastewater treatment efficiencies of MFCs.

Wastewater	COD (mg L^{-1})	COD removal rate (%)	Oil concentration (mg L^{-1})	Oil removal rate (%)	pH
Influent	450.0 ± 30.0	–	18.500 ± 0.500	–	7.10 ± 0.10
Effluent of MFC-M	63.2 ± 5.0	85.96 ± 1.10	3.604 ± 0.200	80.52 ± 1.10	3.90 ± 0.10
Effluent of MFC-P	60.7 ± 5.0	86.67 ± 1.10	2.958 ± 0.200	84.01 ± 1.10	3.88 ± 0.10
Effluent of MFC-D	67.5 ± 5.0	85.00 ± 1.10	4.220 ± 0.200	77.19 ± 1.10	4.01 ± 0.10
Effluent of MFC-M + P	107.4 ± 5.0	76.13 ± 1.10	6.311 ± 0.200	65.88 ± 1.10	5.87 ± 0.10
Effluent of MFC-M + D	81.4 ± 5.0	81.91 ± 1.10	4.268 ± 0.200	76.93 ± 1.10	5.29 ± 0.10
Effluent of MFC-P + D	58.5 ± 5.0	87.00 ± 1.10	2.672 ± 0.200	85.56 ± 1.10	3.73 ± 0.10
Effluent of MFC-M + P + D	96.3 ± 5.0	78.60 ± 1.10	4.645 ± 0.200	74.89 ± 1.10	5.54 ± 0.10

electron transfer performance, a large number of acids and esters was generated (Table 2), so the anolyte pH decreased sharply. The MFC-P + D displayed the highest COD and oil removal rates with a low effluent pH (3.73 ± 0.10), whereas the COD and oil removal rates of the MFC-M + P were the lowest with a much higher pH (5.87 ± 0.10).

To validate the results of the microbial metabolic reactions obtained above, GC–MS was used to analyze the organic components contained in MFC wastewater influent and effluent. The results are presented in Table 2. The organic components of the MFC refinery wastewater influent predominantly included volatile phenols, aromatic hydrocarbons and aliphatic hydrocarbons. However, the relative contents of the volatile phenols and aromatic hydrocarbons in the effluent were lower than the detection limit, and a large quantity of metabolites appeared. The metabolic products from petroleum hydrocarbons degradation predominantly included acetate, lauric acid, palmitic acid and oleic acid, which were mostly from the aliphatic fraction of the hydrocarbons in the anode chamber. These oxygen-containing metabolic products could be produced under a sealed anaerobic chamber attributed to the characteristics of the microbes in MFCs. In the anode chamber, petroleum hydrocarbons could obtain oxygenium from water to generate low molecular organic acids under the electron transfer function of electricigens.

The relative contents of metabolites were quite different in distinct MFCs. In the MFC-P + D inoculated with *Paenibacillus sp.* + *Deinococcus sp.*, the relative metabolite (acids & esters) content was 69.58%, which was observably higher than the other MFCs. We attribute this to the microbial synergistic effect of these two strains. In MFCs inoculated with *Microbacterium sp.* together with other strains, because of the microbial antagonistic effects of *Microbacterium sp.*, the metabolic reaction of petroleum hydrocarbons was inhibited, resulting in higher concentrations of hydrocarbons and fewer detected metabolic products (acids and esters).

3.6. Microbial metabolic characteristics of single microbial strains and their influence on microbial synergistic and antagonistic effects

Detailed studies using GC–MS indicate that acids and esters from the metabolic degradation of petroleum hydrocarbons include mainly acetate, lauric acid, palmitic acid, and oleic acid in the MFC anode chamber. To study the influence of single microbial strains on the degradation characteristics of petroleum hydrocarbons and the influence of metabolites on electricity generation performances, acetate, lauric acid, palmitic acid and oleic acid (all at 100 mg L^{-1}), successively were added as substrates into the MFCs. The influences of the metabolites on the MFC voltage output are presented in Fig. 3a–g.

Table 2
The changes of relative contents of organic components in wastewater.

MFCs	Volatile phenol and aromatic hydrocarbon	Aliphatic hydrocarbon (%)	Acids and esters (%)
Influent	45.41%	54.59	–
Effluent of MFC-M	–	39.12	60.88
Effluent of MFC-P	–	30.90	69.10
Effluent of MFC-D	–	41.82	58.18
MFC-M + P	–	71.01	28.99
MFC-M + D	–	55.72	44.28
MFC-P + D	–	28.77	69.58
MFC-M + P + D	–	67.40	32.60

The addition of acetate observably inhibited the voltage output performances of MFCs inoculated with *Microbacterium sp.*, *Deinococcus sp.*, *Paenibacillus sp.* + *Microbacterium sp.*, *Paenibacillus sp.* + *Deinococcus sp.* and *Microbacterium sp.* + *Deinococcus sp.* + *Paenibacillus sp*. These results indicate that microbial strains such as *Microbacterium sp.* and *Deinococcus sp.* could use petroleum hydrocarbons and primary metabolites as substrates to generate acetate but they could not degrade them effectively. Therefore, the exogenous addition or intrasystem accumulation of acetate restrained the metabolic activity of *Microbacterium sp.* and *Deinococcus sp.* thereby decreasing the petroleum hydrocarbon degradation rate. The depressant effect of acetate on *Microbacterium sp.* was so great that the voltage output could not recover to the original level even when the other metabolite was added. Conversely, the addition of acetate improved the voltage output performance of the MFC inoculated with *Paenibacillus sp.*, indicating that *Paenibacillus sp.* may degrade acetate well. However, when MFCs were inoculated with *Paenibacillus sp.* and the other two microbial strains, the acetate degradation function of *Paenibacillus sp.* was inhibited by the other two microbial strains.

The depressant effect of lauric acid on the voltage output of each MFC was weak, but it observably improved the voltage outputs of MFCs inoculated with both *Deinococcus sp*. and *Deinococcus sp.* + *Microbacterium sp*. This indicates that *Deinococcus sp*. could degrade lauric acid well, but *Paenibacillus sp.* and *Microbacterium sp.* could not degrade lauric acid effectively. Moreover, *Paenibacillus sp.* inhibited lauric acid degradation by *Deinococcus sp*., so when lauric acid was added, the voltage output of the MFC inoculated with *Deinococcus sp.* + *Paenibacillus sp*. did not increase.

Large amount of palmitic acid was contained in *Deinococcus sp*., and when the palmitic acid concentration limit was reached, the metabolic activity of *Deinococcus sp*. was inhibited. Therefore, the voltage output of the MFC inoculated with *Deinococcus sp*. was restrained by the addition of palmitic acid. However, the addition of palmitic acid observably improved the voltage output of all the other MFCs, which indicated that both *Paenibacillus sp.* and *Microbacterium sp.* exhibited good palmitic acid degradation performance that could not be inhibited by *Deinococcus sp.*

The addition of oleic acid could inhibit the voltage output of MFCs inoculated with *Deinococcus sp*. and *Deinococcus sp.* + *Microbacterium sp.*, suggesting that *Deinococcus sp*. could use petroleum hydrocarbons and the primary metabolites to generate oleic acid. Moreover, the addition of oleic acid improved the voltage output of MFCs inoculated with *Paenibacillus sp.*, *Paenibacillus sp.* + *Microbacterium sp.* and *Paenibacillus sp.* + *Deinococcus sp*. This

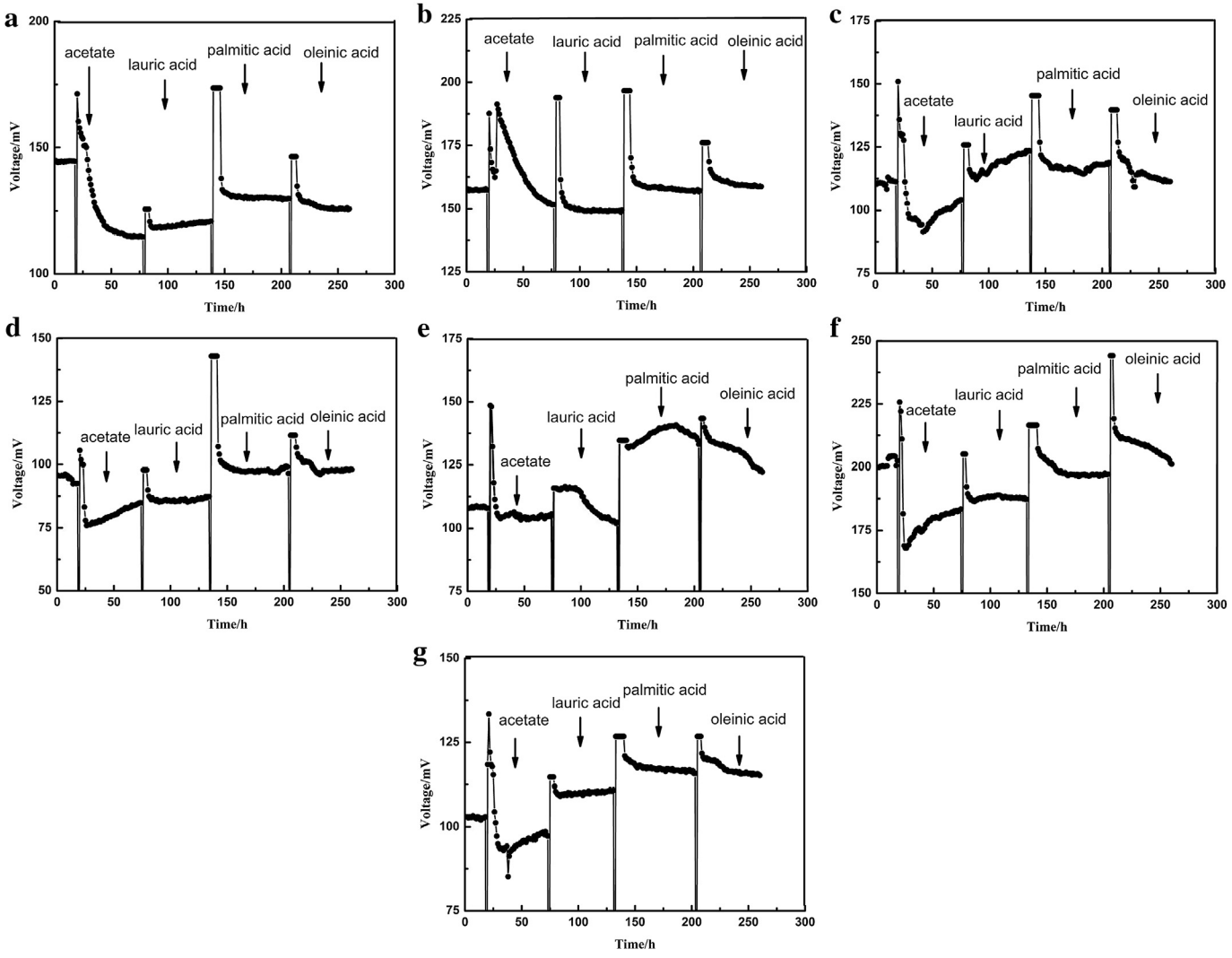

Fig. 3. a. The influence of metabolites on an MFC inoculated with *Microbacterium sp.* (MFC-M). b. The influence of metabolites on an MFC inoculated with *Paenibacillus sp.* (MFC-P). c. The influence of metabolites on an MFC inoculated with *Deinococcus sp.* (MFC-D). d. The influence of metabolites on an MFC inoculated with *Microbacterium sp.* + *Paenibacillus sp.* (MFC-M + P). e. The influence of metabolites on an MFC inoculated with *Microbacterium sp.* + *Deinococcus sp.* (MFC-M + D). f. The influence of metabolites on an MFC inoculated with *Paenibacillus sp.* + *Deinococcus sp.* (MFC-P + D). g. The influence of metabolites on an MFC inoculated with *Microbacterium sp.* + *Paenibacillus sp.* + *Deinococcus sp.* (MFC-M + P + D).

indicated that *Paenibacillus sp.* could degrade oleic acid well and was not inhibited by the other two microbial strains.

Microbacterium sp. successfully degraded petroleum hydrocarbons and the primary metabolites to generate acetate and exhibited a good palmitic acid degradation performance, which could not be inhibited by *Deinococcus sp*. *Deinococcus sp*. could produce acetate, palmitic acid and oleic acid by consuming the petroleum hydrocarbons and the primary metabolites. Moreover, it degraded lauric acid extremely well, but the lauric acid degradation was inhibited by *Microbacterium sp.* and *Paenibacillus sp*. *Paenibacillus sp.* degraded acetate, palmitic acid and oleic acid well, but acetate degradation was inhibited by the other two microbial strains.

These results provide further insight to explain how the microbial synergistic and antagonistic effects influence MFCs. Because the primary function of *Deinococcus sp*. was to degrade petroleum hydrocarbons and their primary metabolites to produce simple fatty acids and esters, whereas *Paenibacillus sp.* degraded the simple fatty acids and esters, they could cooperate to degrade substrates and generate electricity in MFCs and improve the wastewater treatment and electricity generation performances. As presented in Sections 3.4 and 3.5, the MFC-P + D inoculated with these two microbial strains exhibited the best electricity generation and wastewater treatment performances (the maximum power density output was102.93 mW m^{-3}, the COD removal rate was 87.00 ± 1.10% and the oil removal rate was 85.56 ± 1.10%). However, the existence of *Microbacterium sp.* inhibited the bio-functions of *Deinococcus sp*. and *Paenibacillus sp*., so the performances of MFCs inoculated with *Microbacterium sp.* and other two microbial strains were much poorer, and the worst performance was observed in the MFC-M + P inoculated with *Microbacterium sp.* + *Paenibacillus sp* (the maximum power density output was 24.93 mW m^{-3}, the COD removal rate was 76.13 ± 1.10% and the oil removal rate was 65.88 ± 1.10%).

4. Conclusions

Microbial synergistic and antagonistic effects indeed exist among different microbial strains in MFCs. Synergistic effects were observed between *Paenibacillus sp.* and *Deinococcus sp.*, and *Microbacterium sp.* exhibited an antagonistic effect against *Paenibacillus sp.* and *Deinococcus sp*. The microbial synergistic or antagonistic effects significantly influenced the electricity generation and wastewater treatment performances of MFCs directly. The MFC

inoculated with *Paenibacillus sp.* + *Deinococcus sp.* exhibited the strongest performance due to the synergistic effect, where the maximum power density output reached 102.93 mW m^{-3}, and the oil removal rate was 85.56 ± 1.10%. However, due to the antagonistic effect to the other microbial strains, MFCs inoculated with *Microbacterium sp.* exhibited the weakest performance especially the MFC-M + P inoculated with *Microbacterium sp.* + *Paenibacillus sp.*, where the maximum power density output was 24.93 mW m^{-3}, and the oil removal rate was 65.88 ± 1.10%. The characteristics of petroleum hydrocarbons degraded by different microbial strains are notably distinct. These results provide insight into how the microbial synergistic and antagonistic effects influence MFCs.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jpowsour.2013.11.066.

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